Solvent Free Enzymatic Transesterification of Crude Jatropha Oil in Packed Bed Reactor

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Abstract—**In this study, we produce biodiesel from jatropha oil through enzymatic synthesis in a re circulated packed bed reactor (PBR). A solvent free system by three stepwise addition of one equivalent molar methanol in each step was chosen for this study. The enzyme was *Lipozyme IM*, a 1,3 regiospecific immobilized lipase from *Mucor miehei*. Initially, we scrutinized the appropriate amount of lipase by varied the lipase dosage from 5% to 20% based on Jatropha oil weight. Constant operating condition for all experiment was set to temperature of 45° C, PBR flow rate of 5 ml/min and 3 molar equivalent of methanol. The highest biodiesel yield of 54% was obtained at 10% of lipase dosage. We have also examined the effect of methanol addition time by conduct an experiment with one equivalent molar of methanol. The maximum conversion was found at 24 hours. However, a subsequent experiment carried out based on profile from those experiment, could only gave a biodiesel yield of 51%. Afterwards, we observe the lipase stability in PBR solvent free system.**

Keywords- Solvent Free, Packed Bed Reactor, Lipozyme IM, Biodiesel, Enzymatic Transesterification

I. INTRODUCTION

Biodiesel production through enzymatic synthesis from non food feedstock has been drawing many attentions recently. The major drawback of enzymatic synthesis application for biodiesel industry is the high price of the enzyme and lipase inactivation by excess methanol. The inactivation of lipase is probably due to methanol-oil immiscibility [1] and distortion of excess methanol towards the essential water layer that stabilizes the immobilized enzyme [2]. Repeated use of immobilized lipase is expected could minimize the cost of high price enzyme. Whereas methanol inhibition could be work out by various techniques in solvent and solvent free system. In our study, a solvent free system for transesterification of Jatropha oil were carried out in Packed Bed Reactor by using *Lipozyme IM* as biocatalyst.. We decide on a solvent free system for our study, as it is more environmental friendly and doesn't involve any separation or purifications process. Jatropha oil is a non food oil feedstock. The oil from Jatropha seed contain of toxic substance with high oil content. The main problem for utilization of Jatropha oil is the high free fatty acid, which makes this less preferable for biodiesel production through chemical transesterification. So far to our knowledge, it is not a problem in biodiesel production by enzymatic transesterification. This have been proved by research from Shah.et.al, 2007, where increasing free fatty acid content in Jatropha oil will gave similar final alkyl ester (biodiesel) yield. There are several commercially available enzymes for biodiesel production, either in free or immobilized form. The most slender lipase use for this application are Novozyme 435 (from *Candida Antarctica*), Lipozyme TLIM (from *Thermomyces lanuginosus*) and Lipozyme RM IM (from *mucor miehei*). *Lipozyme IM* is a 1, 3 regiospecific lipase immobilized on macroporous ion exchanger resin support which is suitable for continuous Packed Bed Reactor[4]. The Packed Bed Reactor (PBR) have been employed in many industrial heterogenous catalyst application, for the reason that it allows reuse of the enzyme without the need of a prior separation and suitable for long term use without damages the biocatalyst [5,6]. Confidently, it should be more efficient for biodiesel production by using immobilized lipase.

II. MATERIAL AND METHODS

A. Material

Crude Jatropha oil was from Indonesia and immobilized lipase from M*ucor miehei* (*Lipozyme IM*), a novo nordisk trademark, was purchased from Sigma Aldrich, Malaysia. Methanol, Methyl Ester standard and Hexane were from Sigma Aldrich Malaysia.

B. Methods

Enzymatic synthesis: Enzymatic synthesis of biodiesel from *Jatropha curcas* oil was carried out in Packed Bed Reactor (PBR). The PBR consist of two glass columns i.e. internal packed column and jacketed column with length of 25 cm and 20 cm, respectively. *Lipozyme IM* is packed into glass column with bed length of 10 cm, followed by inserting glass wool in the middle and end of bed column. Reaction mixture was flowed from 50 ml glass stirred vessel with agitation of 600 rpm through re circulated PBR with constant flow of 5 ml/min. Methanol was added three-stepwise to the glass vessel. Methanol to oil molar ratio was fixed to 3:1. Temperature in jacketed column and glass vessel was controlled at 45° C by circulated water bath. Product samples were mixed with n-hexane prior to analyze with GC.

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Biodiesel analysis : Methyl ester yield was analyzed by Gas chromatograph-FID (GC 6890) from Agilent. The injector and detector were set to 250° and 280° C, respectively. A DB-23 capillary column was used with oven temperature programming. The injection volume for methyl ester standard and product samples was 1µl.

III. RESULT AND DISCUSSION

A. *Effect of Lipase Dosage*

Lipase dosage is importance parameter in enzymatic synthesis of biodiesel where it could affect the process mechanism as well as final biodiesel yield. In our study, lipase dosage was varied from 5% to 20% based on oil weight with bed length varied from 4 cm to 15 cm, respectively. The reaction was carried out for 24 hours. As can be seen in Figure.1, the biodiesel yield increased with the increasing lipase dosage up to 10%. Doubling the lipase dosage to 20% could not enlarge the biodiesel yield.

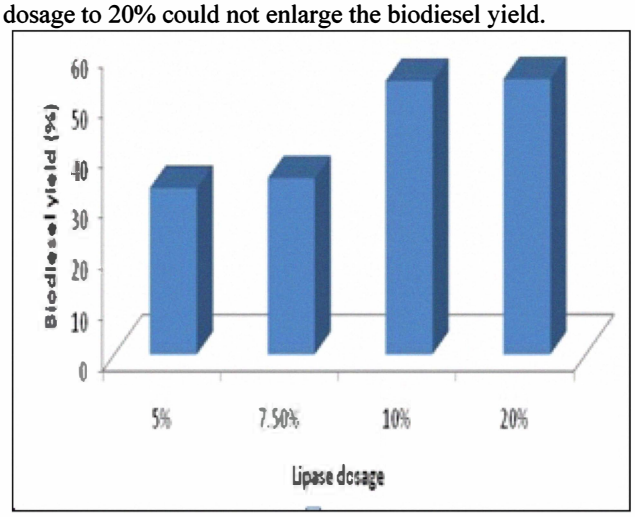

Figure 1. Effect of various lipase dosages on biodiesel yield

The highest biodiesel yield obtained with 10% of *lipozyme IM* was 54.% .The yield from this study was lower than theoretically maximum completed conversion could be achieved (66.6%) with 1, 3 specific lipase. This probably due to formation of glycerol which tends to adsorb on to immobilized support. In PBR system mixture of product and reactants were re circulated repeatedly during the process. Hence, the glycerol was accumulated on the immobilized support.

B. *Effect of time in three stepwise addition of Methanol*

Three stepwise additions is one of techniques in solvent free system which could avoid inhibition effect of methanol. In three stepwise addition of methanol, the molar amount added to the reaction is equal. Hence, it is crucial to decide the accurate time for methanol addition. A set of experiment with one molar methanol added to the reaction was performed. The product sample was taken every hour up to 24 hours of reaction. The profile is given in Figure.2.

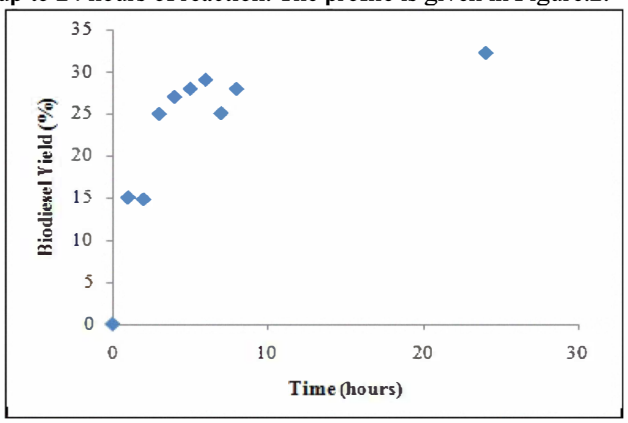

Figure 2. Enzymatic transesterification profile with one equivalent molar of methanol

It can be examined from the figure that biodiesel formed quickly after the first hour than it gradually rise up to 5 hours. Afterwards, yield slowly climbs up to 32% in 24 hours (almost 96% from theoretical conversion of 33.3%). Therefore, a subsequent research was carried out by adding methanol at initial and every 24 hours. Reaction time was extended to 72 hours and the samples were taken for each 24 hours. The profile was shown in Figure. 3.

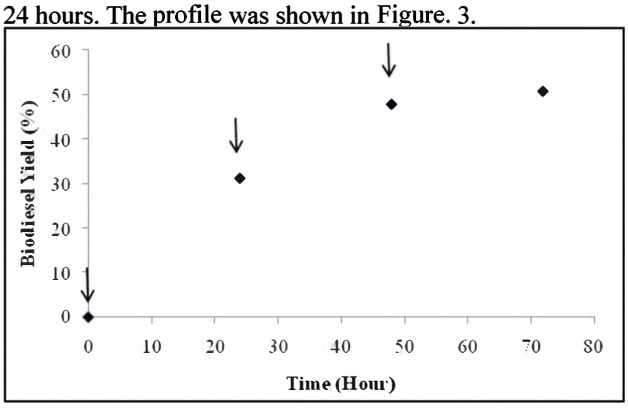

Figure 3. Enzymatic transesterification profile with three stepwise addition of 3 molar of methanol

The biodiesel yield was gradually improved in each addition of methanol time (arrows) up to 72 hours where it reached 51% of biodiesel yield (Figure. 3). Hence, it can be concluded that lengthen the reaction time in PBR solvent free system did not affect the yield.

C. *Stability of Lipozyme IM in PBR*

The stability of the lipase in PBR was examined by directly introducing fresh Jatropha oil and without removal of *lipozyme IM* in each cycle.

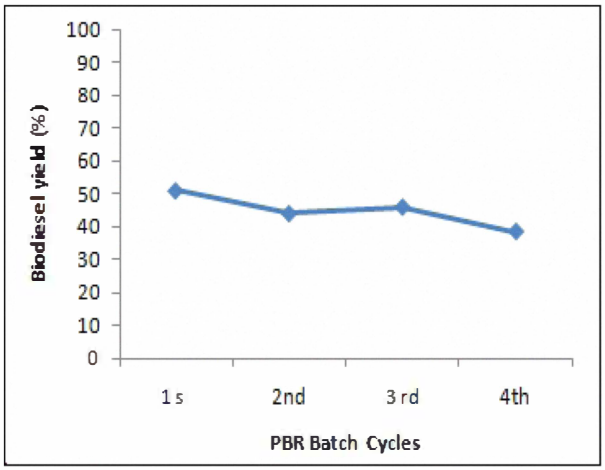

Figure 4. Stability of *Lipozyme IM* in 4 batch cycles

It is shown in Figure.4, biodiesel yield was slowly descend from first to 2nd batch cycle, constant at 3rd and continue to decrease at 4th batch cycle. Glycerol adsorption onto immobilized support of enzyme was greater by the increasing number of batches cycle. This glycerol effect is more likely due to mass transfer limitation in immobilized enzymes than enzyme inhibition [7].

IV. CONCLUSION

A study on solvent free system in biodiesel synthesis from Jatropha oil by using *Lipozyme IM* was performed in Packed Bed Reactor (PBR). Effect of lipase dosage, time for methanol addition in three stepwise and lipase stability were observed. The highest methyl ester yield of 54% achieved from lipase dosage of 10% within 24 hours of reaction. Extending the reaction time to 72 hours would not increase the biodiesel yield in each cycle during lipase stability study. The low yield of biodiesel is most probably due to accumulation of glycerol adsorb onto immobilized support of lipase. Therefore, further research is necessary to evaluate technique that can handle accumulation of glycerol but at the same time will not affect the reaction system.

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